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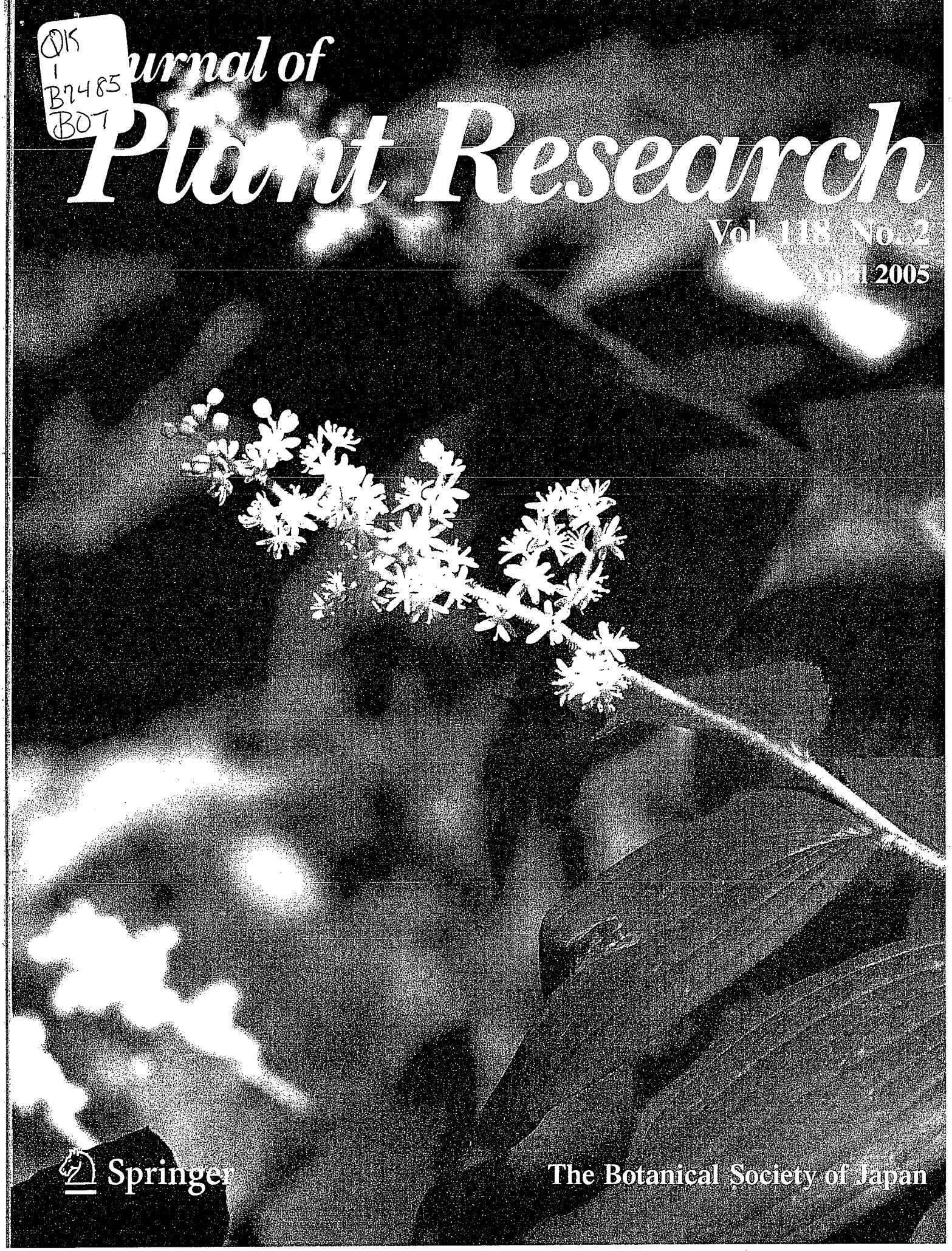

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ORIGINAL ARTICLE

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Developmental morphology of the Asian one-leaf plant, *Monophyllaea glabra* (Gesneriaceae) with emphasis on inflorescence morphology

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Abstract We examined the developmental morphology of the tropical Asian one-leaf plant *Monophyllaea glabra*, which is believed to have diverged first in the phylogenetic tree of the genus. The embryo within the seed consists of two cotyledons and a hypocotyl with no shoot or root apical meristems. The endogenous root meristem is formed nearer the hypocotyl end than in other examined *Monophyllaea* species. One of the cotyledons grows to form the macrocotyledon by means of the basal meristem. The groove meristem arises between the anisocotyledons, shifts toward the macrocotyledon, and is transformed to the inflorescence apex, which produces inflorescence axes in the axils of all ventral bracts of two rows, and secondary inflorescences in the axils of the lower dorsal bracts of the other two rows. The macrocotyledon may act as a ventral bract for the first inflorescence axis at the reproductive stage. This organization suggests that a common ancestor of *Monophyllaea* and *Whytockia* with decussate inflorescences diverged in one direction to become *Monophyllaea* and in another to become *Whytockia*.

Key words Anisocotyly · Groove meristem · Inflorescence · *Monophyllaea glabra* · Monophylly · One-leaf plant

Introduction

One-leaf plants in the *Monophyllaea* and *Streptocarpus* genera of the Gesneriaceae have a curious and unique aerial vegetative part consisting of a single large leaf and a long petiole-like stalk. The *Monophyllaea* is entirely unifoliate (Burtt 1963), but some *Streptocarpus* species show caulescent, rosulate and unifoliate growth forms (Hilliard and Burtt 1971). The origin of the single leaf from one of two cotyledons in the *Monophyllaea* (Ridley 1906) and *Streptocarpus* (Caspary 1858; Crocker 1860) has attracted the interest of physiologists, geneticists and morphologists (e.g., Hill 1938; Lawrence 1958; Bell 1991). Since each genus is attributed to a different tribe of the subfamily Cyrtandroideae (i.e., *Monophyllaea* to Klugieae and *Streptocarpus* to Didimocarpeae) (Burtt 1963; Smith et al. 1997), each genus is believed to have acquired the unifoliate form independently (Burt 1994).

From their developmental study of *Streptocarpus*, Jong and Burtt (1975) interpreted the body construction of one-leaf plants based on the phyllomorph concept. A phyllomorph is a unit comprising a lamina and stalk, and the one-leaf plant is interpreted as a cotyledonary phyllomorph. The phyllomorph is formed by three meristems; the basal meristem contributes to accrescent lamina growth, the petiolode meristem causes stalk elongation, and the groove meristem initiates inflorescences (Jong and Burtt 1975; Jong 1978; Imaichi et al. 2000).

Application of exogenous plant hormones (Rosenblum and Basile 1984) or antagonists of hydroxyproline-protein (Rauh and Basile 2000, 2003) produce a stem-like structure in monophyllous *Streptocarpus*, suggesting [along with anatomical examination (Jong 1978)] that the groove meristem is an embryonic shoot apical meristem (SAM) with delayed rather than suppressed development. On the other hand, Tsukaya (1997) and Cronk and Möller (1997) claimed that unifoliate morphology is similar in some ways to the loss-of-function mutation of the *Arabidopsis thaliana* shoot meristemless (stm) mutant lacking a SAM (Barton and Poethig 1993).

There have been few morphological studies of *Monophyllaea* since Oehlkers (1923). Tsukaya (1997) recently confirmed the lack of a SAM in seedlings of *Monophyllaea horsfieldii*, proposing cotyledon competition as a hypothesis explaining their unequal development. Imaichi et al. (2001) showed that three meristems are involved in the develop-

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ment of *M. singularis* as in *Streptocarpus*. With a few exceptions, most species of *Monophyllaea* have inflorescence aggregations that are more complex than those of *Streptocarpus* at the base of the macrocotyledon (Burtt 1963). In a comparative morphology of *M. horsfieldii* and *Whytockia*, a sister genus of *Monophyllaea* (Mayer et al. 2003), Weber (1975, 2003) suggested that the *Monophyllaea* inflorescence might be derived from the reproductive shoot of *Whytockia* by shortening and reduction.

Molecular phylogenetic analysis of *Monophyllaea* species, with *Whytockia* as the outgroup, using ITS1 between the 18S rRNA and 5.8S rRNA coding regions and ITS2 between the 5.8S rRNA and 26S rRNA coding regions, indicated that *Monophyllaea glabra* Ridley with a small macrocotyledon and a short inflorescence probably diverged first in the *Monophyllaea* tree (H. Setoguchi and M. Ayano, unpublished data). This study examines the developmental morphology of *M. glabra* with emphasis on its inflorescence, and investigates the evolution of monophylly and the inflorescence.

Materials and methods

Seeds and young-to-mature plants of *Monophyllaea glabra* were collected in Ban Nai Sra, Khao Thong, Thailand, in January 2000. Voucher specimens are housed in the Forest Herbarium, Department of National Parks, Wildlife and Plant Conservation, Bangkok (BKF), and the University of Tokyo Herbarium (TI). Seedlings were cultivated in pots filled with vermiculite soils in a phytotron at Japan Women's University at 25°C under plant growth lamps at 17 μmol m^{-2} s^{-1} with a photoperiod of 12-h light and 12-h dark. Plants were also grown in a greenhouse of the Botanical Gardens, the University of Tokyo.

For anatomical study, plants at various developmental stages were fixed in FAA (50% ethyl alcohol, acetic acid, formalin) for over 24 h, dehydrated through a graded ethyl-alcohol series, embedded in LKB Historesin (glycol methacrylate; Leica, Heidelberg, Germany), and cut into 2.5–3.0-μm sections. The sections were stained with a solution of safranin, toluidine blue and orange G (Jernstedt et al. 1992). The mold-cast technique was used for scanning electron microscopy (SEM) observation of cotyledons. Molds made from dental paste (Provil novo Light base + catalyst, Heraeus Kulzer, Hanau, Germany) were filled with an epoxy adhesive and incubated at 60°C for 1 h to obtain casts (Jernstedt et al. 1992). Formed casts were sputter-coated with platinum-palladium and observed at 7–10 kV in a Hitachi S-800 SEM. To observe inflorescence structures, fixed materials were dehydrated in an ethyl-alcohol series, dried using a Hitachi critical-point dryer (HCP-2), coated with platinum-palladium, and photographed under a dissecting binocular microscope (Leica M 240).

Results

Ontogeny and gross morphology

The seeds of *Monophyllaea glabra* are small, elongate (approx. 0.3 mm) and exalbuminous. Germination started 3 days after sowing with the hypocotyl appearing first through the ruptured seed coat, followed by the developing cotyledons. The cotyledons are isocotylous until reaching a length of 0.5 mm. At the end of the isocotylous stage, the first root appears from the distal end of the hypocotyl. During the subsequent anisocotylous stage, one cotyledon (microcotyledon) stops growing while the other (macrocotyledon) continues growing up to a length of more than 5 cm. As the macrocotyledon lamina enlarges, the hypocotyl also elongates, giving rise to a long petiolode (up to 5 cm). The first root does not elongate further, and many adventitious roots arise from the petiolode to support the aerial plant body.

Under phytotron cultivation, the plants grow rapidly and mature 2 months after seed sowing. When the macrocotyledon becomes 4–5 cm in length, it generally forms a cluster of inflorescence axes at the lamina base (Fig. 1a). The macrocotyledon continues growing even after inflorescence formation starts, forming new inflorescences continuously. When one plant reaches the reproductive stage in one pot, the other plants in the same pot start initiating inflorescences, although the macrocotyledons are less than 4 cm long; the smallest inflorescence-bearing macrocotyledon was only 2 cm long. This is consistent with field observations in Kao Thong, Thailand, where nearly all plants in a single population bear inflorescences irrespective of age, and macrocotyledon lengths varies considerably from 2 to 13 cm (Fig. 1a). The mechanism underlying this simultaneous flowering is unknown.

The inflorescence is comprised of several inflorescence axes arranged in two rows along the macrocotyledon midrib (Fig. 1b). It ascends towards the tip of the macrocotyledon, bearing inflorescence buds at the tip. Hence, the inflorescence is dorsiventral toward the macrocotyledon. In dorsal view, the inflorescence axes have a zigzag arrangement, or apparently sympodial branching (Fig. 1c). The inflorescence has two kinds of lanceolate scale-like bracts on the dorsal and ventral sides. The dorsal bracts (red in Fig. 1c) occur at every point of the zigzag branching of the inflorescence and are arranged in two close rows. The ventral bracts (blue in Fig. 1c) occur at the base of each inflorescence axis on the ventral-lateral side of the inflorescence and are hardly visible from the dorsal view. Dorsal bracts are smaller than ventral bracts.

Each inflorescence axis forms many bractless flower pairs (see Fig. 4i) in an acropetal direction, and is comparable to the pair-flowered cyme of *Monophyllaea horsfieldii* (sensu Weber 1976). After several inflorescence axes become well developed, secondary inflorescences arise in the microcotyledon axils as well as in the axils of dorsal bracts of the primary inflorescence axes (Fig. 1d). The secondary inflorescences also branch in a manner similar to

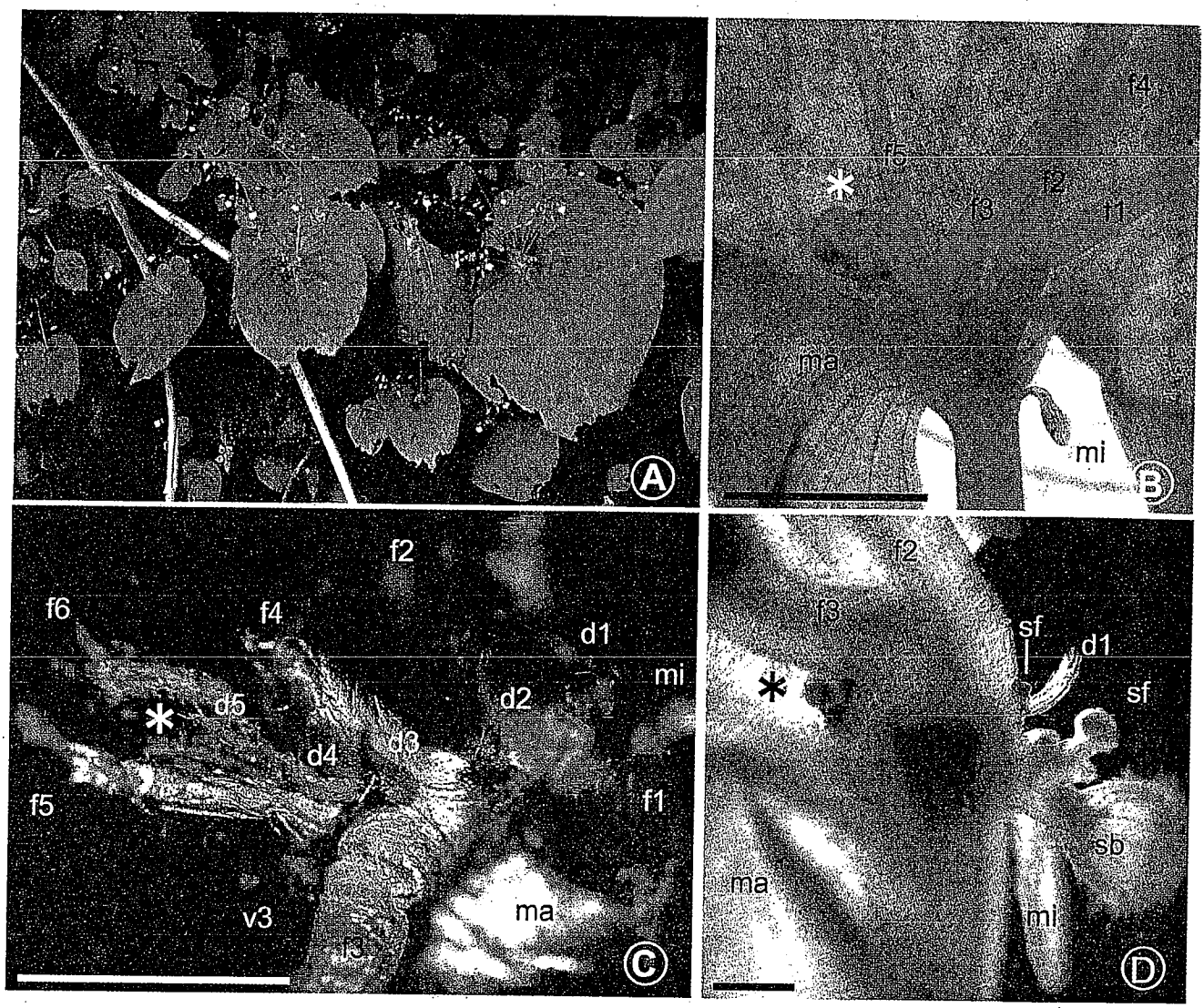

Fig. 1a–d. *Monophyllaea glabra*. Asterisks in **b–d** indicate apices of primary inflorescences. **a** Natural population comprising flowering plants of various sizes in Kao Thong, Thailand. **b–d** Inflorescence architecture. **b** Close-up of primary inflorescence with several axes in two rows. Fixed material is used. **c** Dorsal view of primary inflorescence showing dorsal bracts (artificially *red*) and ventral bract (*blue*). **d** Basal portion of primary inflorescence and secondary inflorescences arising at bases of microcotyledon and first dorsal bract. *d1–d5* First to fifth dorsal bracts, *f1–f6* first to sixth inflorescence axes, *ma* macrocotyledon, *mi* microcotyledon, *sb* bract of secondary inflorescence, *sf* secondary inflorescence, *v3* third ventral bract. *Bars* **b** 5 mm, **c** 2.5 mm, **d** 0.5 mm

that of the zigzag branching of the primary inflorescence. Consequently, the inflorescence aggregation of *M. glabra* is complex and composed of primary and secondary inflorescences.

Embryo and root development during germination

The embryo within the seed consists of two cotyledons and a hypocotyl with no SAMs or root apical meristems (RAM)(Fig. 2a). The hypocotyl end is often attached by the suspensor remnant (Fig. 2b). In median longitudinal section, both cotyledons are four to five cells long and three to four cells thick including the protoderm and procambium; the hypocotyl is seven cells long and seven to eight cells thick (Fig. 2a). Embryo cells are full of starch grains. A procambial strand runs through the hypocotyl and both cotyledons. There are four ranks of smaller cells distal to the end of the procambial strand at the distal tip of the hypocotyl (Fig. 2b). The outer, first and second ranks each consist of two cells, and the inner, third and fourth ranks consist of one cell each.

During germination, the hypocotyl length doubles due to an increase in cell number from 7 to 16 cells along the axis, and subsequent cell enlargement in an upward direction. Cell enlargement starts at the proximal end of the hypocotyl, resulting in a thick hypocotyl end with truncate base (Fig. 2c). Enlarged cells in the hypocotyl tip become less stained due to a decrease in the number of starch grains caused by metabolic consumption increasing; rhizoidal hairs grow all over the hypocotyl-tip, including the centrally located first-rank cells (Fig. 2c). Meanwhile, third- and

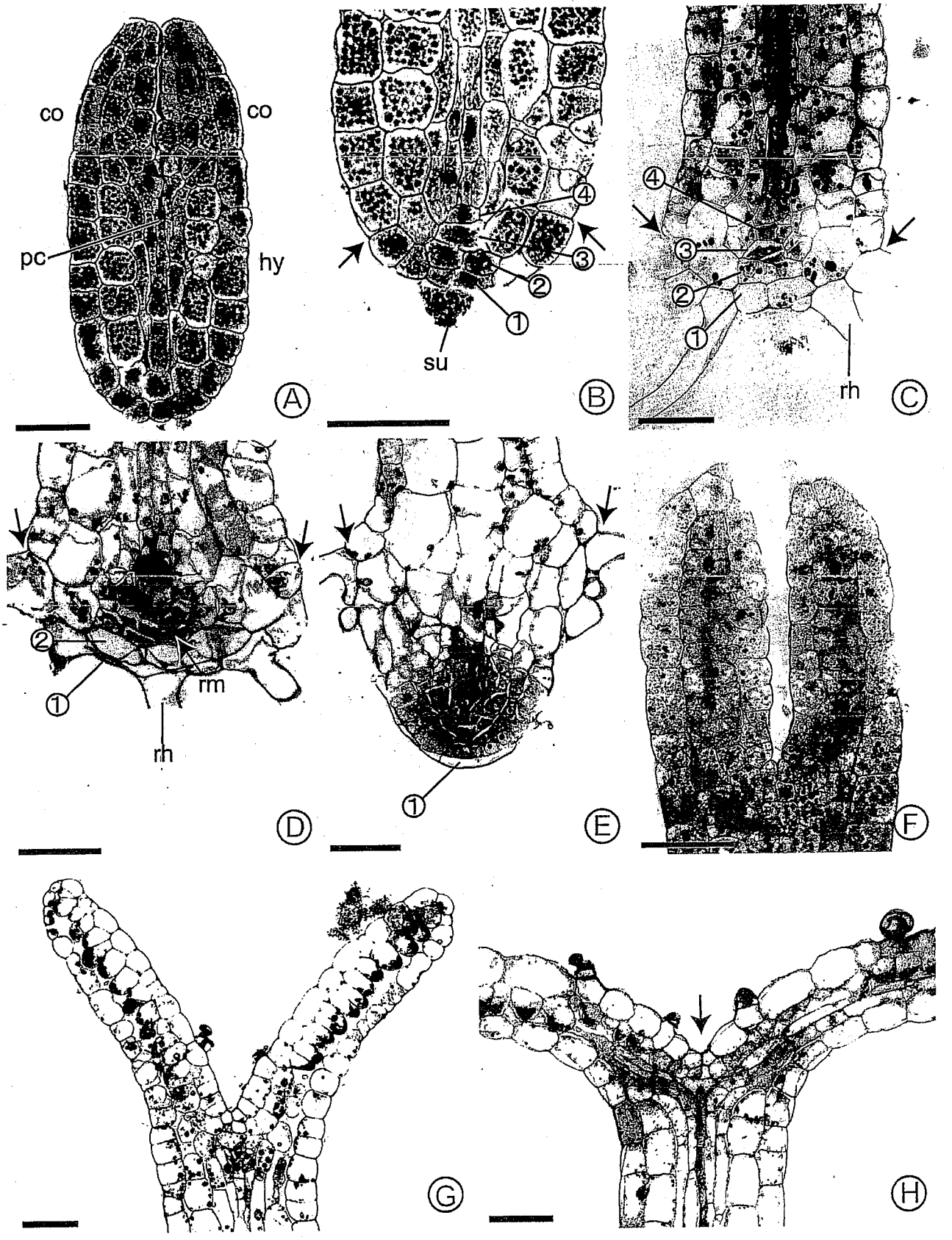

Fig. 2a–h. Longitudinal sections (*LS*) showing seedling development. *Arrows* in **b–e** indicate the future boundary between hypocotyl and first root. **a** Embryo removed from seed, lacking shoot apical meristem (SAM) and root apical meristem (RAM). **b** Neighboring LS from **a** showing basal half of hypocotyl. Four ranks of cells (1–4) are distal to the procambial end. **c** Enlarged hypocotyl tip with root initial cells derived from third-rank (*3*) and fourth-rank (*4*). **d** Developing hypocotyl tip with root meristem developed within inner two cell layers (*1*, *2*). **e** Protruding root still covered by remnant of the first-rank cells (*1*) of hypocotyl. **f** Elongating cotyledons and distal portion of hypocotyl. **g** Unfolding cotyledons retaining small mesophyll cells at base. **h** Fully unfolded cotyledons showing absence of meristem in place of SAM (*arrow*). *co* Cotyledon, *hy* hypocotyl, *pc* procambium, *rh* rhizoidal hair, *rm* root meristem, *su* suspensor remnant. *Bars* 50 μm

fourth-rank cells distal to the procambial end remain small with densely stained cytoplasm and become the root initial cells (Fig. 2c).

Shortly after the root initial cells start division, the surrounding cells, including terminal procambium cells, become meristematic to contribute to the root meristem. The early root meristem cells are covered by surface and subsurface cells derived from first- and second-rank and adjacent cells (Fig. 2d). At this early stage, the root meristem axis is often oblique to the hypocotyl axis. Subsequently, the second-rank cells give rise to the root protodermal cells by repeated anticlinal divisions (Fig. 2e). As the root meristem develops, the first-rank cells and surrounding surface cells with rhizoidal hairs stretch and finally rupture due to the downward root growth. As a result, rhizoidal hairs remain only on the periphery of the hypocotyl end (Fig. 2e). In young seedlings, this portion with persisting rhizoids demarcates the boundary between the hypocotyl and first root.

Cotyledon unfolding and establishment of anisocotyly

During germination, the protodermal and ground meristem cells of the cotyledons, like those of the hypocotyl, double in number due to anticlinal cell divisions, becoming 10–12 cells long (Fig. 2f). The cells start enlarging in the basipetal direction from cotyledon tip to base. As a result, the distal part of the unfolding cotyledons is composed of larger differentiating cells, while small protodermal and mesophyll cells with small intercellular spaces remain at the base of both cotyledons (Fig. 2g). This basipetal cell enlargement is also detected in SEM micrographs. A SEM image of the abaxial protoderm of an unfolding cotyledon with a lamina length of 0.25 mm, shows that cells of the distal two-thirds are relatively large and undulating, while those of the proximal one-third are smaller and rectangular with straight (not undulating) cell walls (Fig. 3a). The adaxial protodermal cells also remain equally small and rectangular at the base of more-developed cotyledons (Fig. 3b).

During unfolding, the bases of both cotyledons change from obtuse to round, eventually becoming truncate in the late isocotylous stage (Figs. 3a–c). In young cotyledons with an obtuse base, the abaxial surface has about 16 radial (proximo-distal) cell files, some with two subfiles produced by T-divisions (Fig. 3a). In the more-developed cotyledons with a round base, although cell enlargement occurs as described, the same number of radial cell files (about 16) is found at the adaxial epidermises (Fig. 3b). In fully unfolded cotyledons with a truncate base, 20 or so radial cell files are recognized at the abaxial (Fig. 3c) and adaxial epidermises (data not shown). Consequently, the number of radial cell files at the surfaces increase by only five during the entire unfolding, suggesting that cotyledon unfolding is due mainly to cell enlargement after early cell increment. Therefore, the change in the lamina-base shape during the isocotylous stage may be driven by this development.

At the end of the isocotylous stage, surface cells at the base change shape and size between the cotyledons. The basal cells are elongate in one isocotyledon but small and rectangular (Fig. 3d) in the other. These small cells are often grouped in somewhat elongated merophytes, indicating that they are a product of recent basal cell division. The basal meristem is expanding increasingly during macrocotyledon growth (Fig. 3f). Due to its activity, the macrocotyledon base again becomes as round as in the young cotyledons (Fig. 3e), before becoming cordate at maturity (Fig. 1a). On the other hand, the microcotyledon hardly grows at all, and becomes spatulate (Fig. 1b).

Groove meristem development and inflorescence initiation

The isocotylous seedlings have no visible meristem at the site of the SAM (Fig. 2h). Instead, in median longitudinal section, two small surface cells usually occupy the bottom between the two adjacent cotyledons (Figs. 2g, h). In the early anisocotylous stage, one of these two central surface cells begins dividing (Fig. 4a). Concurrently, the subsurface cells below the dividing surface cells become meristematic (Fig. 4a). Associated with macrocotyledon growth, the area of the dividing small cells expands (Fig. 4b) and becomes the flat groove meristem (Fig. 4c), showing the tunica-corpus configuration typical of the angiosperm SAM (Fig. 4c). In SEM micrographs, the groove meristem with epicuticular deposits on the surface occurs close to the base of the growing macrocotyledon, apart from the microcotyledon and obliquely elongate toward the microcotyledon (Fig. 4d). At this stage, the groove meristem is probably transformed into the inflorescence apex.

The inflorescence apex widened transversely to the intercotyledonary axis, simultaneously forming the first primary inflorescence axis and dorsal bract on the microcotyledon side (Fig. 4e). Consequently, three meristematic mounds appear: (1) the first inflorescence axis, (2) the first dorsal bract, and (3) the inflorescence apex (Fig. 4f). A longitudinal section through mounds (1) and (3) (f1 and asterisk in Fig. 4e) shows two continuous tunica-like cell layers between the two mounds (Fig. 4g), suggesting that the first inflorescence axis is derived exogenously from the inflorescence apex. Later, the inflorescence apex produces another bract (ventral bract) on the side opposite the first dorsal bract (Fig. 4h). Although it forms first among ventral bracts, this ventral bract subtending the second inflorescence axis, is called the second ventral bract.

In a top view of the more-developed inflorescence (Fig. 4i), the more-developed second ventral bract and the second dorsal bract are formed nearly in front of the first dorsal bract. In Fig. 4i, the second inflorescence axis primordium is hidden under the hyponastically growing second ventral bract. Concurrently, the inflorescence apex enlarges again toward the macrocotyledon (Fig. 4i) and subsequently initiates the third inflorescence axis. Serial oblique longitudinal sections of a slightly more developed inflorescence show that as the third dorsal bract develops (Fig. 5a), the meristem of the third inflorescence axis forms in the axil of the third ventral bract (Figs. 5a, b) and the third ventral bract begins forming adjacent to the inflorescence meristem (Fig. 5c).

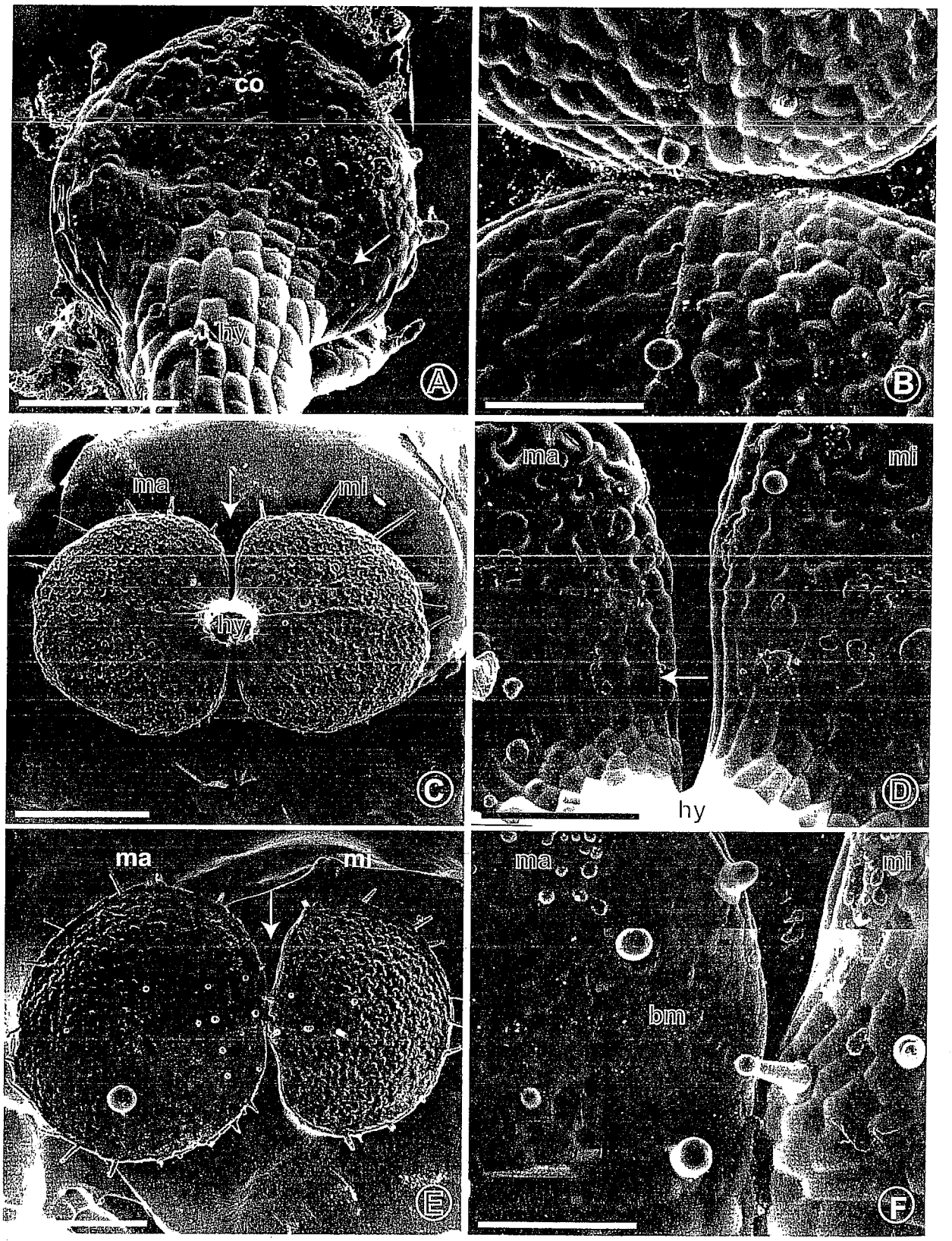

Fig. 3a–f. Scanning electron microscopy (SEM) images of cotyledons at successive development stages, showing establishment of basal meristem. Abaxial (**a, c, d**) and adaxial (**b, e, f**) views. **a** Expanding cotyledons with small cells (*arrow*) at base of radial cell files. **b** Bases of unfolded isocotyledons 0.3 mm long. **c** Apparent isocotyledons 0.6 mm long with truncate bases; *arrow* site magnified in **d**. **d** Basal parts of cotyledons showing elongate merophytes (*arrow*) consisting of small cells in future macrocotyledon. **e** Anisocotyledons with macrocotyledon 0.85 mm long and round at base; *arrow* site magnified in **f**. **f** Bases of cotyledons showing basal meristem comprising small cells in macrocotyledon. *bm* Basal meristem, *co* cotyledon, *hy* hypocotyl, *ma* macrocotyledon, *mi* microcotyledon. *Bars* **a**, **b**, **d**, **f** 100 μm; **c**, **e** 500 μm

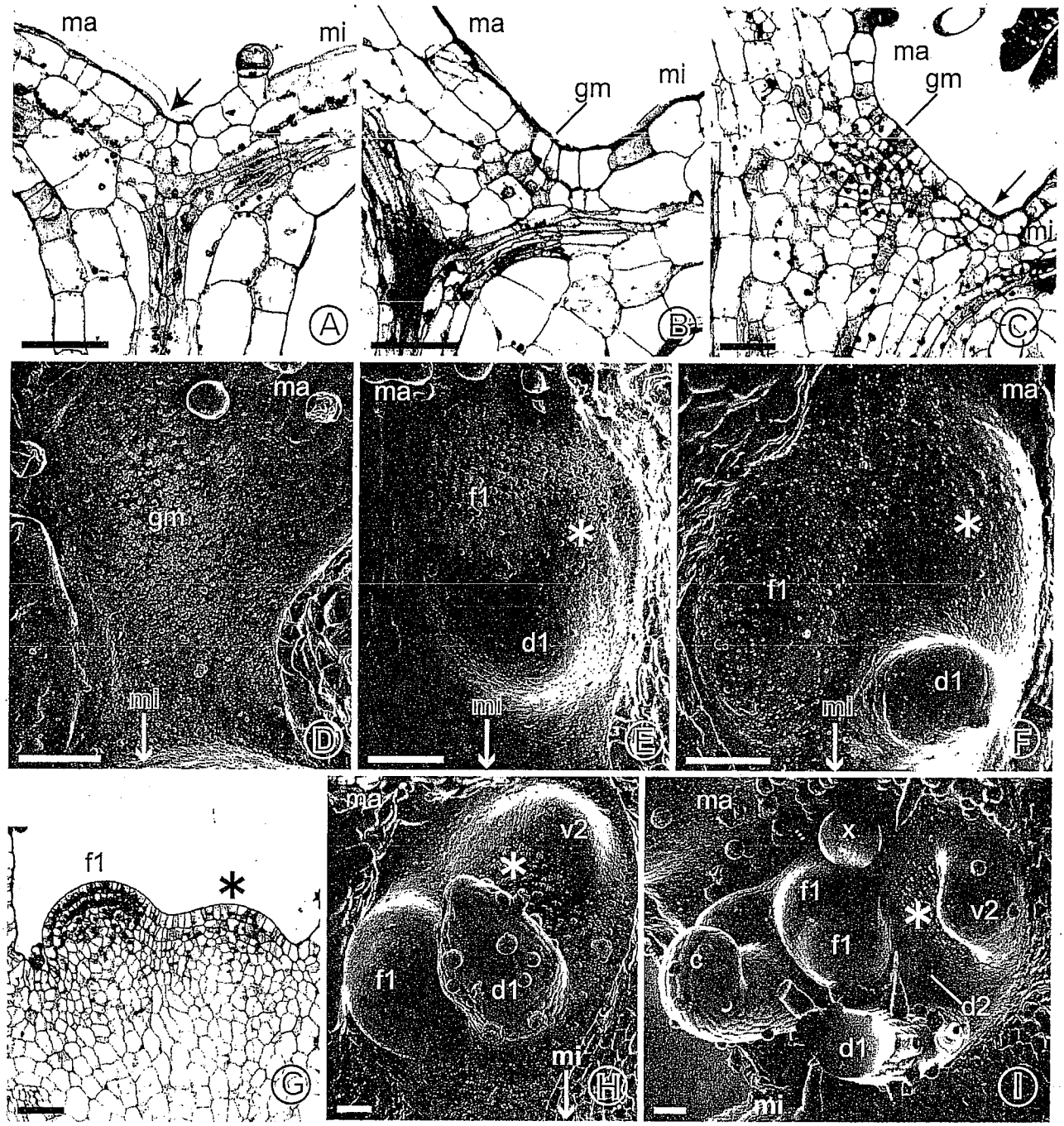

Fig. 4. Development of groove meristem (**a–d**) and primary inflorescence (**e–i**). **a–c**, **g** LS; **d–f**, **h**,**i** SEM. *Asterisks* in **e–i** Apices of primary inflorescences (inflorescence meristems). **a** Anticlinal division of small cell (*arrow*) close to macrocotyledon. **b** Groove meristem at early stage. **c** Developed groove meristem showing tunica-corpus like configuration. Note another group of apparently meristematic cells in the axil of the microcotyledon (*arrow*). **d** Groove meristem obliquely enlarged along long axis of cotyledons (vertical in figure) at stage slightly older than **c**. **e** Inflorescence apex, meristem of first inflorescence axis and dorsal bract. **f** More-developed inflorescence. **g** Inflorescence apex with primordium of first inflorescence axis. Section of inflorescence apex is submedian. **h** Further-developed inflorescence. Note absence of ventral bract subtending first inflorescence axis. **i** Further-developed inflorescence with second inflorescence axis under *v2* (not seen). One calyx lobe of the first pair of flowers is visible but the others are not shown. The first apex of the first inflorescence axis (either one of two mounds labeled with *f1*) forms the next pair of flowers (the *cross* indicates an artifact). *c* Calyx lobe of flower, *d1–d2* first to second dorsal bract, *f1* first inflorescence axis, *gm* groove meristem, *ma* macrocotyledon, *mi* microcotyledon, *v2* second ventral bract. *Bars* 50 μm

Thereafter, the inflorescence apex regularly produces primordia of inflorescence axes together with dorsal and ventral bract pairs (Fig. 5d). Importantly, the dorsal bracts do not subtend any inflorescence axes (but see below). The more-developed inflorescence elongates parallel to the macrocotyledon (Fig. 5e) with the inflorescence apex at the top (Figs. 5f, g) and never terminates with an inflorescence axis. Consequently, the inflorescence does not undergo the

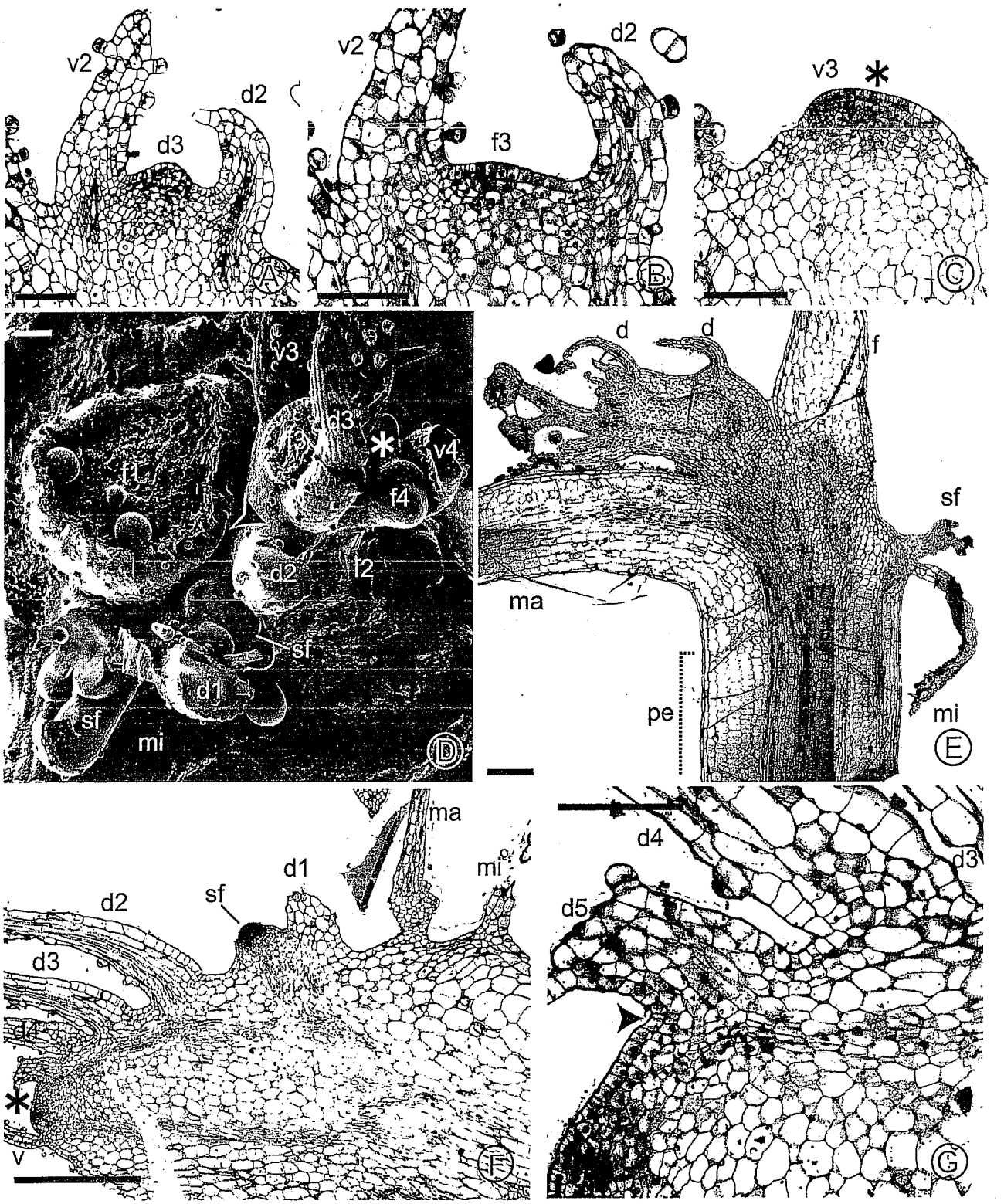

Fig. 5a–g. Further development of primary inflorescence. **a–c, e–g** LS, **d** SEM. *Asterisks* in **c, d, f** Inflorescence apex. **a–c** Selected serial LS of inflorescence slightly older than that in Fig. 4i. Third dorsal bract (**a**), third inflorescence axis (**b**), and third ventral bract (**c**) starting to develop. Meristem of second inflorescence axis not shown. **d** Primary inflorescence with three of four inflorescence axes (*f1–f3*), second ventral bract (*v2*) removed. Inflorescence axes are each accompanied by dorsal and ventral bracts (second ventral and fourth dorsal bracts are not seen). Secondary inflorescences initiate in axils of the first dorsal bract and microcotyledon; *arrowhead* bulge of secondary inflorescence in axil of second dorsal bract. **e** Primary inflorescence with five to six inflorescence axes and secondary inflorescence in microcotyledon axil. **f** Further-developed inflorescence. Note secondary inflorescence apex in axil of first dorsal bract. **g** Magnified figure of apical portion of inflorescence in **f**. Inflorescence apex (*bottom left*) extends to base (*arrowhead*) of youngest dorsal bract. *f, f1–f4* Inflorescence axis and first to fourth inflorescence axis; *d, d1–d5* dorsal and first to fifth dorsal bract; *ma* macrocotyledon; *mi* microcotyledon; *pe* petiolode meristem; *sf* secondary inflorescence; *v, v2–v4* ventral and second to fourth ventral bract. *Bars* **a–d, g** 100 μm; **e** 500 μm

sympodial branching suggested by its zigzag construction, but most likely undergoes monopodial branching.

After the primary inflorescence development described above, two kinds of secondary inflorescences develop. One type is initiated in the axils of the dorsal bracts relatively early compared to development of the primary inflorescence (Figs. 5d, f). The origin of the secondary inflorescence apex can be traced to peripheral cells of the primary inflorescence apex (Fig. 5g). Before such secondary inflorescence development, the other type of secondary inflorescence starts forming in the axil of the microcotyledon (Fig. 5d, e). The future initial cells of this secondary inflorescence are likewise found in the axil of the microcotyledon just as the groove meristem is established (Fig. 4c).

The secondary inflorescences, especially those at the microcotyledon and the first three dorsal bracts, develop rapidly after initiation, and branch several times to form inflorescence axes. As a consequence, the secondary inflorescences plus the primary inflorescence establish an inflorescence complex. However, the first-bract arrangement differs from that of the primary inflorescence because the first inflorescence axis of the secondary inflorescence is subtended by a relatively large bract (Figs. 1d, 6a–c). This contrasts with the first axis of the primary inflorescence, which is not subtended by a bract (Fig. 4h). We do not know whether this bract is either equivalent to the missing ventral bract at the first inflorescence axis of the primary inflorescence, or is another kind of bract.

Both primary and secondary inflorescence axes grow to form pairs of flowers from the apical meristem (Fig. 4i). At maturity, the inflorescence axis comprises an axis and about 10 pairs of flowers with no bracts.

As described above, the petiole-like stalk below the lamina was distinctly long in *M. glabra*. Unlike the petiolode of *Streptocarpus* species, no apparent intercalary meristem is formed near the groove meristem (Fig. 4c). Instead, an intercalary meristem consisting of files of prism-like cells is formed in the hypocotyl under the microcotyledon and contributes to petiolode elongation (Fig. 5e).

Discussion

Origin of root meristem

Like other one-leaf plants, including unifoliate *Streptocarpus* species, the embryo within the seed of *Monophyllaea glabra* has neither SAM nor RAM. The RAM differentiates later, during or after germination. This raises the question of whether embryonic RAM development is delayed or perfectly arrested. In other words, is the first root formed at the hypocotyl tip a primary root or an adventitious root?

The first root in the *Monophyllaea* has been described as exogenous (Oehlkers 1923) and (recently) as endogenous in *Monophyllaea singularis* (Imaichi et al. 2001). The RAM in *M. singularis* originates from cells deep in the hypocotyl tip and ruptures a few outer layers of the hypocotyl tip as the root protrudes. However, our results for *M. glabra* do not fully agree with prior observations because the RAM is initiated from just one cell layer inside the hypocotyl tip. This manner of root initiation is similar to that of the unifoliate *Streptocarpus grandis* (Imaichi et al. 2000).

Embryological data are needed to clarify the nature of the first root. Despite scanty data on the Cyrtandroideae, some results for *Streptocarpus* spp. (Alimova and Yakovlev 1982) suggest that the first root is derived from the hypophysis at the globular embryo stage. Our unpublished embryological data on *Monophyllaea glabra* indicate that the root meristem is similarly derived from the hypophysis, suggesting that the first root of one-leaf plants may be a radicle (embryonic root) in which development is delayed until seedling development. To discuss the homology of the first root, we need more extensive data on the embryology and seedling morphology of all the Cyrtandroideae.

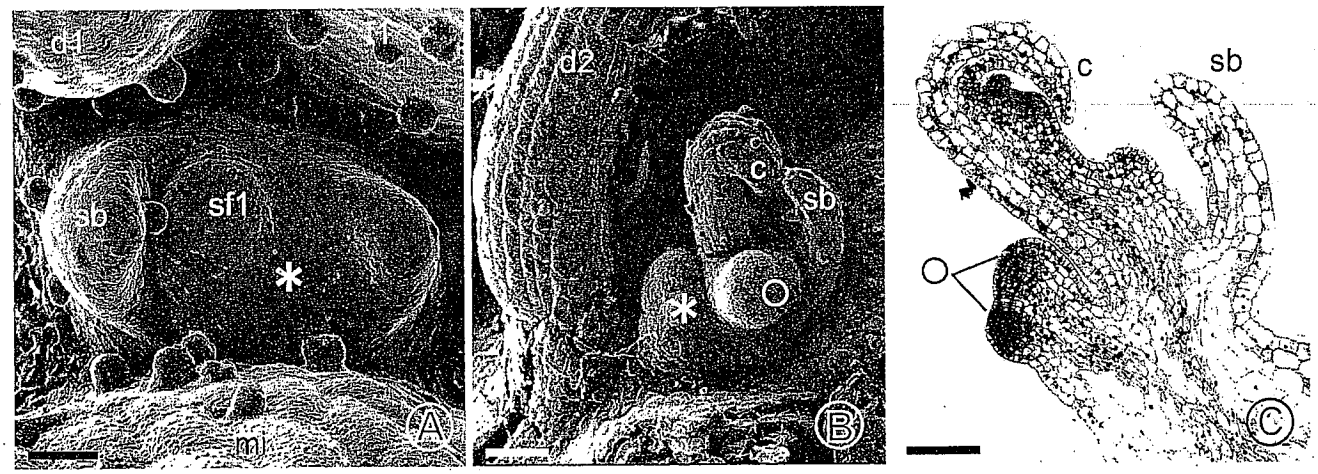

Fig. 6. Development of secondary inflorescence. **a, b** SEM; **c** LS. *Asterisks* in **a, b** Secondary inflorescence apices. **a** Young secondary inflorescence in microcotyledon axil. **b** Secondary inflorescence in axil of second dorsal bract. The mound labeled with a *circle* is the meristem of the secondary inflorescence axis. **c** Secondary inflorescence in axil of third dorsal bract. Two mounds (indicated by *circle*) are probably a pair of flowers. *c* Calyx lobe, *d1–d2* first to second dorsal bract, *fl* first inflorescence axis, *mi* microcotyledon, *sb* bract on secondary inflorescence, *sfl* first inflorescence axis of secondary inflorescence. *Bars* **a** 50 μm; **b, c** 100 μm

Origin of basal meristem

Another long-standing question is the origin of the basal meristem giving rise to the macrocotyledon. There are two current hypotheses: (1) The basal meristem is produced by a failure to switch off cell division at the appropriate time during cotyledon growth; (2) the basal meristem is a new meristem that appears at the cotyledon base (Imaichi et al. 2000). As evidence of DNA synthesis due to meristem activity in *Monophyllaea horsfieldii*, BrdU incorporation occurs equally at the base of both cotyledons soon after germination, but disappears from the microcotyledon (Tsukaya 1997). The meristematic activity remaining at the base of the other cotyledon during basipetal differentiation likely gives rise to the basal meristem in the accrescent macrocotyledon of *M. glabra*, like in *M. singularis* and *Streptocarpus grandis* (Imaichi et al. 2000, 2001). Recent experiments using application of exogenous hormones produced equal expansion of both cotyledons of *S. wendlandii* and *M. glabra*, suggesting that a developmental phase change from the macrocotyledon to the foliage leaf occurs when the anisocotylous stage starts (Nishii et al. 2004). This phase change seems to be related to establishment of the basal meristem. Our results with *M. glabra* suggest that cell division becomes much more active when the basal meristem is established. This is probably related to the phase change to the foliage leaf or an equivalent phase in *M. glabra* as proposed by the phase-change hypothesis (Nishii et al. 2004).

Inflorescence development with implications for evolution of monophylly

In a discussion on the evolution of monophylly in relation to the taxis of bracts and inflorescence axes, Weber (1976, 2003) regarded the inflorescence of the *Monophyllaea* as a shortened shoot of the sister plant *Whytockia* (Mayer et al. 2003). The hypothetical ancestral shoot is caulescent with decussate leaves each subtending inflorescence axes; two adjacent rows of larger leaves bear inflorescence axes, but the other two smaller leaves are "sterile" and the axillary inflorescence branches for the smaller leaves are already completely reduced (aborted). This stage is seen in *Whytockia*. The larger "plus" leaves and smaller "minus" leaves (Weber 1975) of *Whytockia* are considered homologous to the inflorescence-subtending ventral bract and the dorsal bract of *M. horsfieldii*, respectively (Weber 2003). Both leaves are then reduced to equal small ones, and a final slight positional change occurs in the minus leaves.

Our results show that the taxis of *Monophyllaea glabra* bracts and inflorescence axes is the same as in *M. horsfieldii* (compare Fig. 7 with Abb. 11 in Weber 1975 and Fig. 3 in Weber 2003), suggesting that the species probably present a common morphology for the *Monophyllaea*. Because the *M. glabra* ventral bract subtends an inflorescence axis and the dorsal bract does not, the ventral and dorsal bracts seem equivalent to Weber's (2003) plus and minus leaves, respectively. Furthermore, the inflorescence apex produces several inflorescence axes as the SAM generally produces lateral branches. The groove meristem is probably a SAM due to its between-cotyledonary appearance and tunica-corpus histology, but whether it is a plumular SAM remains undecided. These results partly support Weber's hypothesis that the *Monophyllaea* inflorescence corresponds to a shortened *Whytockia* reproductive shoot. However, the first inflorescence axis of *M. glabra* does not follow the hypothesis, because it is not subtended by a ventral bract. Therefore, it is possible that the *M. glabra* macrocotyledon replaces the first ventral bract functionally, an interpretation that is consistent with our observed developmental

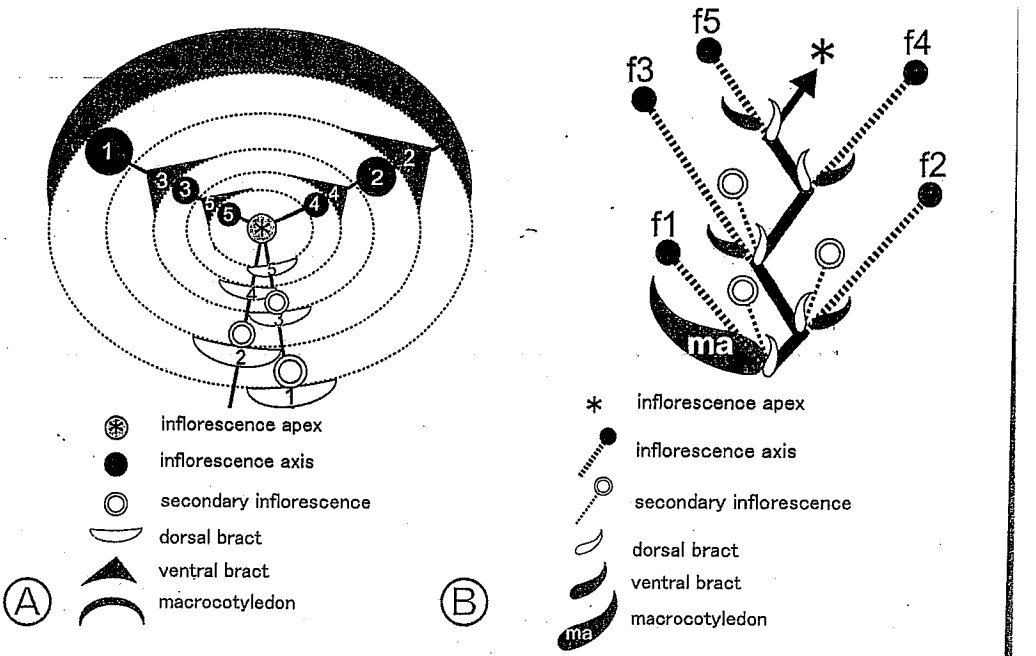

Fig. 7. Plan diagram of taxis and arrangement of inflorescence axes and two types of bracts (**a**) and dorsal-view diagram of inflorescence branching (**b**). The first inflorescence axis seems to be subtended by the macrocotyledon

pattern. The groove meristem appears close to the base (or in the axil) of the macrocotyledon as interpreted by Weber (1975, 2003), and becomes the inflorescence apex, which produces the first inflorescence axis associated with the developing macrocotyledon. If this interpretation is correct, the development of *M. glabra* is an integrated process in which the macrocotyledon has an additional role as a ventral bract subtending an inflorescence axis at the next reproductive stage. Evolution of the one-leaf plant inflorescence may have been associated with loss of shoot organogenesis in the vegetative phase if *Whytockia* has an ancestral morphology similar to that of other angiosperms. *Whytockia* has a vegetative phase with foliage leaves not subtending axillary inflorescences between the embryonic phase and the reproductive phase. Therefore, it may not be an immediate ancestor to the *Monophyllaea* morphologically.

Our observations showed that the four rows of bracts in *Monophyllaea glabra* can produce inflorescence axes or secondary inflorescences. This inflorescence organization is reminiscent of a decussate phyllotaxis proposed for the ancestor to *Monophyllaea* like that of most Klugieae. Weber (1975, 2003) hypothesized that the ancestor evolved into *Whytockia* and then into *Monophyllaea* associated with changes in the phyllotaxis and "fertility" of the bracts (i.e., a change to two adjacent inflorescence-subtending bracts and to two other sterile bracts). However, the evolution of *Whytockia* with inflorescence-subtending bracts in two rows into *Monophyllaea*, in particular *M. glabra* with the above inflorescence organization and the *Monophyllaea* basal species, may not be tenable. Based on our results, we propose that a common ancestor for both genera with "fertile" bracts in four rows diverged to *Monophyllaea* and *Whytockia*.

In conclusion, in *Monophyllaea glabra* the first root is endogenous in the hypocotyl tip of a developmentally retarded embryo. The macrocotyledon develops due to the basal meristem that appears at the phase change from the cotyledon to the foliage leaf or an equivalent state. The groove meristem arises between the cotyledons in a young seedling with no obvious SAM and becomes the inflorescence apex close to the base of the macrocotyledon. The meristem produces a subdecussate inflorescence, which may be a primitive morphological state. The monophylly of *Monophyllaea* seems to be reduced from the more general morphology of the common ancestor to *Whytockia* with a diffuse inflorescence developed on an anisocotylous seedling.

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